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(FILE 'HOME' ENTERED AT 09:39:25 ON 26 NOV 2002)

FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 09:39:42  
ON 26 NOV 2002

L1 2289 S EOSINOPHIL? (5A) (CLASSIF? OR IDENTIF? OR DISTINGUISH?)  
L2 2104 S EOSINOPHIL? (P) (CYTOMET?)  
L3 131 S L1 (6P) L2  
L4 46 DUP REM L3 (85 DUPLICATES REMOVED)

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ACCESSION NUMBER: 96351616 MEDLINE  
DOCUMENT NUMBER: 96351616 PubMed ID: 8742174  
TITLE: **Identification of eosinophils by flow cytometry.**  
AUTHOR: Thureau A M; Schylz U; Wolf V; Krug N; Schauer U  
CORPORATE SOURCE: Institut fur Immunologie der medizinischen Fakultat, Universitat Rostock, Germany.  
SOURCE: CYTOMETRY, (1996 Feb 1) 23 (2) 150-8.  
Journal code: 8102328. ISSN: 0196-4763.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199610  
ENTRY DATE: Entered STN: 19961022  
Last Updated on STN: 19961022  
Entered Medline: 19961010

AB A flow **cytometric** method to **identify** and characterize **eosinophils** in lysed whole blood samples was established. A gating protocol was applied that in the first step uses the high autofluorescence and the high sideward scatter of **eosinophils**. In the second step, **eosinophils** were differentiated from neutrophils by lack of CD16 expression or alternatively presence of CD49d expression. **Eosinophils** purified by density gradient centrifugation (purity: 93% **eosinophils** contaminated with 7% neutrophils) were used to evaluate the technique. We were able to **identify eosinophils** added back to lysed whole blood samples and to **identify** partial degranulated **eosinophils** after treatment with secretory IgA and anti-IgA. In addition we were able to show that due to a large overlap of sideward scatter, the technique is applicable to purified normodense as well as hypodense **eosinophils**. In addition, there was a good correlation ( $r = 0.921$ ,  $P < 0.0001$ ) between the percentage of **eosinophils** determined by flow **cytometry** and microscopic evaluation in 81 patients. In patients with atopic dermatitis there was a reasonable correlation between a severity score (SCORAD) and the number of **eosinophils** determined by flow **cytometry** ( $R = 0.6107$ ,  $P = 0.017$ ). Since the technique proved to be able to **identify** activated **eosinophils** bearing the CD69 early activation antigen, the relation between serum creatinine and CD69 expression on peripheral blood **eosinophils** was analysed showing a positive correlation ( $r = 0.4344$ ,  $P = 0.016$ ).

TI **Identification of eosinophils by flow cytometry.**

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by flow **cytometry** ( $R = 0.6107$ ,  $P = 0.0017$ ). Since the technique proved to be able to **identify** activated **eosinophils** bearing the CD69 early activation antigen, the relation between serum creatinine and CD69 expression on peripheral blood **eosinophils** was analysed showing a positive correlation ( $r = 0.4344$ ,  $P = 0.016$ ).

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L4 ANSWER 24 OF 46 MEDLINE DUPLICATE 16  
 ACCESSION NUMBER: 1998101773 MEDLINE  
 DOCUMENT NUMBER: 98101773 PubMed ID: 9440823  
 TITLE: Identification of eosinophils in lysed whole blood using  
 side scatter and CD16 negativity.  
 AUTHOR: Gopinath R; Nutman T B  
 CORPORATE SOURCE: Helminth Immunology Section, Laboratory of Parasitic  
 Diseases, National Institutes of Health, Bethesda, Maryland  
 20892, USA.. rgopinath@pop.niaid.nih.gov  
 SOURCE: CYTOMETRY, (1997 Dec 15) 30 (6) 313-6.  
 Journal code: 8102328. ISSN: 0196-4763.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199802  
 ENTRY DATE: Entered STN: 19980226  
 Last Updated on STN: 19980226  
 Entered Medline: 19980218

AB The **identification of eosinophils** in lysed whole blood  
 by flow **cytometry** can be problematic, since these cells overlap  
 significantly with the neutrophil cluster on forward scatter versus side  
 scatter plots of whole blood samples. Current methods can be  
 time-consuming when running multiple samples or may compromise yield in  
 the interests of greater accuracy. The use of **eosinophil**  
 purification techniques prior to FACS analysis or sorting as a way of  
 ensuring purity may have unpredictable effects on **eosinophil**  
 activation, leading to questionable data interpretation. Here we describe  
 a simple, single-step method for definition of **eosinophils**  
 utilizing their high side scatter and CD16 fluorescence negativity to  
 differentiate them from neutrophils. The purity of the neutrophil and  
**eosinophil** populations sorted with this gate is close to 100%  
 regardless of the peripheral blood **eosinophil** count, while the  
 population obtained by sorting on a plot of FSC/SSC was a mixture of  
**eosinophils** and neutrophils. We suggest this method as a simple,  
 reproducible, and accurate way of defining **eosinophils** by flow  
**cytometry** for analysis or sorting.

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 purity of the neutrophil and **eosinophil** populations sorted with  
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 plot of FSC/SSC was a mixture of **eosinophils** and neutrophils. We  
 suggest this method as a simple, reproducible, and accurate way of  
 defining **eosinophils** by flow **cytometry** for analysis or  
 sorting.

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L4 ANSWER 2 OF 46 USPATFULL

ACCESSION NUMBER: 2002:212610 USPATFULL

TITLE: High numerical aperture flow cytometer and method of using same

INVENTOR(S): Roche, John W., Scarborough, ME, UNITED STATES  
Hansen, W. Peter, Canaan, NY, UNITED STATES  
Flynn, Harold C., JR., Scarborough, ME, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002113965	A1	20020822
APPLICATION INFO.:	US 2001-969242	A1	20011002 (9)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 2000-507515, filed on 18 Feb 2000, GRANTED, Pat. No. US 6320656		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	LYON & LYON LLP, 633 WEST FIFTH STREET, SUITE 4700, LOS ANGELES, CA, 90071		
NUMBER OF CLAIMS:	15		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	21 Drawing Page(s)		
LINE COUNT:	516		

AB The high numerical aperture flow cytometer of the present invention includes a flow cell and a laser input. The laser input emits a beam of light that is oriented substantially orthogonally to the flow of blood cells through the flow cell such that laser light impinges upon the blood cells as they pass through the flow cell. A portion of the beam from the laser input that impinges upon the blood cells in the flow cell is scattered at a substantially right angle to the beam of laser input ("right angle scatter"). A second portion of the beam from the laser input that impinges upon the cells in the flow cell is scattered at a much lower angle than 90.degree.. This scatter is termed forward scatter light" and is collected on two distinct photo detectors, that represent "forward scatter low" (FSL) which has an angle of from about 1.degree. to about 3.degree., and "forward scatter high" (FSH) which has an angle of from about 9.degree. to about 12.degree. from the orientation of the original beam from laser input. A third photo detector is placed in between these two forward scatter detectors, that is axial with the impinging laser light. This detector measures axial light loss, or light extinction (EXT) which is the sum of all the light that is absorbed and scattered by the blood cells. A right angle scatter light detector is oriented to receive the previously mentioned right angle scatter light. A forward scatter light detector is oriented to capture the previously mentioned forward scatter light oriented different angles from the beam of the laser input.

SUMM [0006] The prior art as indicated in the '497 Patent is unable to **distinguish eosinophils** without utilizing polarized and depolarized light methods, because the cone of light collected is 72.degree. or less, based on the. . .

SUMM [0007] Copending U.S. patent application Ser. No. 09/507,515 discloses a device and method for **distinguishing eosinophils** in a sample of blood cells. The device uses a right angle scatter light detector that is effective to collect. . . collected right angle scattered light into a right angle scattered light signal. This signal is processed by the device to **distinguish eosinophils** from other leukocytes in the sample on the basis of the right angle scattered light signal.

SUMM . . . are not apparent in the prior art. Thus, a lens less light collection system may be used in a flow **cytometer**, which has a much lower numerical aperture, but maintains cluster separation of **eosinophils**. The advantage of this device is that a lower numerical aperture system can be produced more efficiently and more reproducibly. . .

DETD [0044] Referring to FIG. 5, the output of the data from the flow cytometer of the present invention is shown. FIG. 5 has the output of right angle scatter light detector 22 as one axis and the output of low angle forward scatter light detector 24 as the other axis. **Eosinophils** are located to the right of the software threshold line and, as shown in FIGS. 6A, 7A, 8A, and 9A, . . .

DETD . . . 6B, 7A, 7A, 7B, 8A, 8B, 9A and 9B, graphical representations of leukocyte identification is shown, with specific reference to **eosinophil identification**. The data of FIGS. 6A, 7A, 8A, and 9A was employed using the apparatus of the present invention. In FIGS. . . .

CLM What is claimed is:

1. A high numerical aperture flow cytometer, comprising: a flow cell; a laser input, said laser input emitting a beam of light that is oriented substantially orthogonally. . . . angle scattered light into a right angle scattered light signal; and a signal processor, said signal processor being effective to **distinguish eosinophils** from other leukocytes on the basis of said right angle scattered light signal.

L4 ANSWER 4 OF 46 USPATFULL

ACCESSION NUMBER: 2002:137738 USPATFULL  
 TITLE: Dual large angle light scattering detection  
 INVENTOR(S): Altendorf, Eric H., Edmonds, WA, United States  
 PATENT ASSIGNEE(S): University of Washington, Seattle, WA, United States  
 (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6404493	B1	20020611
APPLICATION INFO.:	US 2000-574930		20000519 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1998-169533, filed on 9 Oct 1998, now patented, Pat. No. US 6067157		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	GRANTED		
PRIMARY EXAMINER:	Font, Frank G.		
ASSISTANT EXAMINER:	Stafira, Michael P.		
LEGAL REPRESENTATIVE:	Greenlee, Winner and Sullivan, P.C.		
NUMBER OF CLAIMS:	8		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	12 Drawing Figure(s); 10 Drawing Page(s)		
LINE COUNT:	593		

AB An optical analyzer with a configuration particularly suitable for use with planar liquid sample flow cells is provided comprising a polarized light source and at least two large angle scattered light photodetectors positioned respectively at acute, and right or oblique angles to the incident light beams. Differences in intensities of light measured at the two photodetectors are used to quantify components of the sample.

SUMM . . . Light Scattering: Identification and Separation of Unstained Leukocytes," Acta Cytologica 19:374-377). However, within the granulocytes, SALS and FALS cannot clearly **distinguish** between **eosinophils** and the remaining granulocytes such as neutrophils and basophils.

SUMM . . . Granulocyte WBCs, having an internal structure comprising numerous small granules, exhibit a difference in scattering intensity between the polarizations. In **eosinophils** the granules are birefringent and act to depolarize the scattered light, thereby reducing the difference in scattering intensity between the . . . to distinguish cell types (Terstappen, L.W.M.M. et al. (1988), "Four-Parameter White Blood Cell Differential Counting Based on Light Scattering Measurements," **Cytometry** 9:39-43; de Grooth et al., U.S. Pat. No. 5,017,497; Marshall, U.S. Pat. No. 5,510,267. The depolarization was measured by impinging. . . .

SUMM . . . polarizing filters. This analyzer is especially useful with planar flow cells but can also be used with conventional round flow **cytometers**. The analyzer comprises a polarized light source positioned to produce a light beam which intersects a liquid sample flow in. . . be used with any type of flowing particle, it is particularly suited to a hematology analyzer used to count and **classify** blood cells, and in particular **eosinophils**. Preferably .theta..sub.1 is between about 15.degree. and about 50.degree., more preferably about 30.degree.+-.10.degree., and most preferably about 39.degree.+-.10.degree. where the. . .

L4 ANSWER 5 OF 46 MEDLINE DUPLICATE 1  
 ACCESSION NUMBER: 2002165611 MEDLINE  
 DOCUMENT NUMBER: 21895708 PubMed ID: 11897993  
 TITLE: Expression of FcgammaRIII (CD16) on human peripheral blood eosinophils increases in allergic conditions.  
 AUTHOR: Davoine Francis; Lavigne Sophie; Chakir Jamila; Ferland Claudine; Boulay Marie-Eve; Laviolette Michel  
 CORPORATE SOURCE: Unite de recherche en pneumologie, Centre de recherche de l'Hopital Laval, Institut universitaire de cardiologie et de pneumologie de l'Universite Laval, Sainte-Foy, Quebec, Canada.  
 SOURCE: JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (2002 Mar) 109 (3) 463-9.  
 Journal code: 1275002. ISSN: 0091-6749.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals  
 ENTRY MONTH: 200204  
 ENTRY DATE: Entered STN: 20020319  
 Last Updated on STN: 20020419  
 Entered Medline: 20020418

AB BACKGROUND: Blood **eosinophils** have mRNA for FcgammaRIIIB (CD16) but no or minimal spontaneous CD16 expression. Because IFN-gamma and chemotactic factors induce **eosinophil** CD16 expression in vitro, we postulated that blood **eosinophils** could express CD16.  
 OBJECTIVE: Blood of nonallergic controls and subjects with allergic rhinitis, allergic and nonallergic asthma, or hypereosinophilia of various etiologies were analyzed for leukocyte CD16 surface expression. METHODS: CD16(+) **eosinophils** were identified on the basis of physico-optic characteristics, major basic protein, CD49b expression, and sorting by flow **cytometry** and microscope examination. RESULTS: Subjects with allergic rhinitis and subjects with asthma had higher median percentages of CD16(+) **eosinophils** (8.1% [1% to 48.6%] and 7.3% [1.4% to 31.1%], respectively) than nonallergic controls and nonallergic asthmatics (3% [0% to 11%] and 4.6% [2.9% to 5.1%], respectively). In subjects with hypereosinophilia, CD16(+) **eosinophils** were increased only in a case of drug allergy. When subjects with mild allergic asthma were challenged with a relevant aeroallergen, blood CD16(+) **eosinophils** further increased during or after the late-phase response (6 to 48 hours after challenge; mean +/- SEM, 9.4% +/- 2.5% to 20.0% +/- 3.0%). CD16(+) **eosinophils** expressed more IL-5 receptor but less CD11b and IL-12p35 than did CD16(-) **eosinophils**. CONCLUSION: Upregulation of blood CD16(+) **eosinophils** in allergic conditions and its association with a modified phenotype suggest that CD16 receptor could play a role in **eosinophil** activation in allergy.

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etiologies were analyzed for leukocyte CD16 surface expression. METHODS: CD16(+) **eosinophils** were identified on the basis of physico-optic characteristics, major basic protein, CD49b expression, and sorting by flow **cytometry** and microscope examination. RESULTS: Subjects with allergic rhinitis and subjects with asthma had higher median percentages of CD16(+) **eosinophils** (8.1% [1% to 48.6%] and 7.3% [1.4% to 31.1%], respectively) than nonallergic controls and nonallergic asthmatics (3% [0% to 11%] and 4.6% [2.9% to 5.1%], respectively). In subjects with hypereosinophilia, CD16(+) **eosinophils** were increased only in a case of drug allergy. When subjects with mild allergic asthma were challenged with a relevant aeroallergen, blood CD16(+) **eosinophils** further increased during or after the late-phase response (6 to 48 hours after challenge; mean +/- SEM, 9.4% +/- 2.5% to 20.0% +/- 3.0%). CD16(+) **eosinophils** expressed more IL-5 receptor but less CD11b and IL-12p35 than did CD16(-) **eosinophils**. CONCLUSION: Upregulation of blood CD16(+) **eosinophils** in allergic conditions and its association with a modified phenotype suggest that CD16 receptor could play a role in **eosinophil** activation in allergy.

L4 ANSWER 7 OF 46 USPATFULL

ACCESSION NUMBER: 2001:209587 USPATFULL  
TITLE: High numerical aperture flow cytometer and method of  
using same  
INVENTOR(S): Ferrante, Anthony A., Medford, NY, United States  
Hansen, W. Peter, Canaan, NY, United States  
PATENT ASSIGNEE(S): Idexx Laboratories, Inc., Westbrook, ME, United States  
(U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	<u>US 6320656</u>	B1	20011120
APPLICATION INFO.:	US 2000-507515		20000218 (9)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	GRANTED		
PRIMARY EXAMINER:	Pham, Hoa Q.		
LEGAL REPRESENTATIVE:	Lyon & Lyon LLP		
NUMBER OF CLAIMS:	20		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	13 Drawing Figure(s); 12 Drawing Page(s)		
LINE COUNT:	416		

AB The high numerical aperture flow cytometer of the present invention includes a flow cell and a laser input. The laser input emits a beam of light that is oriented substantially orthogonally to the flow of blood cells through the flow cell such that laser light impinges upon the blood cells as they pass through the flow cell. A portion of the beam from the laser input that impinges upon the blood cells in the flow cell is scattered at a substantially right angle to the beam of laser input ("right angle scatter"). A second portion of the beam from the laser input that impinges upon the cells in the flow cell is scattered at a much lower angle than 90.degree.. This scatter is termed "low angle forward scatter light" and has an angle of from about 2.degree. to about 5.degree. from the orientation of the original beam from laser input. A right angle scatter light detector is oriented to receive the previously mentioned right angle scatter light. A low angle forward scatter light detector is oriented to capture the previously mentioned low angled forward scatter light oriented at about 2.degree. to about 5.degree. from the beam of the laser input.

DETD Referring to FIG. 5, the output of the data from the flow cytometer of the present invention is shown. FIG. 5 has the output of right angle scatter light detector 22 as one axis and the output of low angle forward scatter light detector 24 as the other axis. Eosinophils are located to the right of the software threshold line and, as shown in FIGS. 6A, 7A, 8A, and 9A, . . .

DETD . . . 6B, 7A, 7A, 7B, 8A, 8B, 9A and 9B, graphical representations of leukocyte identification is shown, with specific reference to eosinophil identification. The data of FIGS. 6A, 7A, 8A, and 9A was employed using the apparatus of the present invention. In FIGS. . . .

CLM What is claimed is:

1. A flow cytometer, comprising: a flow cell; a laser input, said laser input emitting a beam of light that is oriented substantially orthogonally. . . angle scattered light into a right angle scattered light signal; and a signal processor, said signal processor being effective to distinguish eosinophils from other leukocytes on the basis of said right angle scattered light signal.

. . . of at least 100.degree.; converting said detected unfiltered right angle scattered light into a right angle scattered light signal; and identifying eosinophils present among said biological cells on the basis of said right angle scattered light signal.

11. A flow cytometer, comprising: a flow cell; a laser input,

said laser input emitting a beam of light that is oriented substantially orthogonally. . . angle scattered light into a right angle scattered light signal; and a signal processor, said signal processor being effective to **distinguish eosinophils** from other leukocytes on the basis of said single right angle scattered light signal.

. . . of at least 100.degree.; converting said detected right angle scattered light into a single right angle scattered light signal; and **identifying eosinophils** present among said biological cells on the basis of said single right angle scattered light signal.

L4 ANSWER 20 OF 46 MEDLINE DUPLICATE 12  
 ACCESSION NUMBER: 1999094253 MEDLINE  
 DOCUMENT NUMBER: 99094253 PubMed ID: 9879644  
 TITLE: Detection of eosinophils in whole blood samples by flow cytometry.  
 AUTHOR: Carulli G; Sbrana S; Azzara A; Minnucci S; Angiolini C; Marini A; Ambrogi F  
 CORPORATE SOURCE: Department of Oncology, University of Pisa, Italy.  
 SOURCE: CYTOMETRY, (1998 Dec 15) 34 (6) 272-9.  
 Journal code: 8102328. ISSN: 0196-4763.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199903  
 ENTRY DATE: Entered STN: 19990402  
 Last Updated on STN: 19990402  
 Entered Medline: 19990324

AB A flow **cytometric** method to detect and study human **eosinophils** in whole blood was established. Normal subjects and patients with various types of **eosinophilia** (hypereosinophilic syndromes, allergic diseases, dermatitis, Hodgkin's Disease, parasitosis) were studied. Whole blood samples were treated for 10 minutes at room temperature with a commercially available reagent (FACS Lysing Solution, Becton Dickinson) which acts both as a fixative and as a lysing agent. **Eosinophils** were **identified** as a granulocytic subpopulation with higher SSC and FSC properties. This cell population was characterized by evident autofluorescence and hypodiploid DNA features after propidium iodide staining. The purity of the **eosinophil** population sorted after electronic gating was close to 100%. A very significant correlation between **eosinophil** counting by our whole blood method and other two assays, namely routine automatic counting by the H\*3 Bayer System and **eosinophil** detection by depolarized SSC, was obtained. The phagocytic properties of **eosinophils** were also studied by means of a commercially available diagnostic kit, thus demonstrating that our method is also suitable for the study of those granulocytic functions which can be evaluated by flow **cytometry**.

AB A flow **cytometric** method to detect and study human **eosinophils** in whole blood was established. Normal subjects and patients with various types of **eosinophilia** (hypereosinophilic syndromes, allergic diseases, dermatitis, Hodgkin's Disease, parasitosis) were studied. Whole blood samples were treated for 10 minutes at room. . a commercially available reagent (FACS Lysing Solution, Becton Dickinson) which acts both as a fixative and as a lysing agent. **Eosinophils** were **identified** as a granulocytic subpopulation with higher SSC and FSC properties. This cell population was characterized by evident autofluorescence and hypodiploid DNA features after propidium iodide staining. The purity of the **eosinophil** population sorted after electronic gating was close to 100%. A very significant correlation between **eosinophil** counting by our whole blood method and other two assays, namely routine automatic counting by the H\*3 Bayer System and **eosinophil** detection by depolarized SSC, was obtained. The phagocytic properties of **eosinophils** were also studied by means of a commercially available diagnostic kit, thus demonstrating that our method is also suitable for the study of those granulocytic functions which can be evaluated by flow **cytometry**.

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